



“On the Influence of certain Natural Agents on the Virulence of the Tubercle-Bacillus.” By ARTHUR RANSOME, M.D., F.R.S., and SHERIDAN DELÉPINE. Received May 1, 1894.

Three years ago Dr. Ransome communicated to the Society the results of some experiments, carried out in concert with Professor Dreschfeld, of Owens College, “On certain conditions that modify the virulence of the bacillus of tubercle.”

The tendency of these researches was to prove “that fresh air and light, and a dry and sandy sub-soil, have a distinct influence in arresting the virulence of the tubercle-bacillus; that darkness somewhat interferes with this disinfectant action; but that mere exposure to light, in otherwise bad sanitary conditions, does not destroy the virus.”

The following table gives the results of similar experiments by ourselves.

Table I.

Experi- ment No.	
3.	1. Rabbit inoculated in peritoneum with fresh sputum. Killed 55 days after. Showed well-marked tubereulosis.
7.	2. Rabbit. Sputum exposed to light and air 45 days in June and July. Showed no tubereulosis after 86 days.
8.	3. Rabbit. Sputum exposed in air-shaft in dusk at the same time. Showed slight tubereulosis after 86 days.
11.	4. Guinea-pig. The same sputum exposed at the same time, in air and light, inoculated under the skin. Showed no distinct tuberele in 80 days.
12.	5. Guinea-pig. Same methods, only in dusk. Showed advanced tubereulosis in 80 days.
58.	6. Guinea-pig. Another sputum exposed in April for 16 days to little or no air, in darkness. Gave well-marked tuberele after 42 days.
59.	7. Guinea-pig. Ditto, ditto.

We have now carried the enquiry a little further, and, amongst other objects, have endeavoured to determine how short a period of exposure to air and light would suffice to destroy the poisonous action of the microbe. We selected guinea-pigs as the most susceptible animals to test this question.

In the first instance pure cultivations of the bacillus were prepared, and were found to be active by frequent inoculations. Small portions of this material were spread in a thin layer, upon pieces of sterilized paper. They were arranged in circles of about 2 mm. in diameter, so as to give every opportunity for the action of the elements. They were then exposed in a glass-room, with free access to air and light, *i.e.*, close to open windows, for diminishing periods of time, viz., 14, 10, 6, 4, and 2 days respectively. Contemporaneous daily records were kept of temperature, maximum and minimum, and of the amount of sunshine taken through the glass roof, by means of one of Negretti and Zambra's sunshine recorders.

The following table (Table II) gives the results of the meteorological observations, but as will be seen presently, only those for the first few days are of importance.

No result from the other papers; the control experiments showing that the bacilli used after this date had lost their virulence. Even the results of Experiments 97 and 98 are doubtful on that account, but Experiment 85 was made with a very virulent specimen, as was proved by the inoculation of two other guinea-pigs, with paper infected with the same quantity of the same cultivation, and kept the same length of time, but not exposed to sunlight. In both these cases advanced tuberculosis was produced in 44 days.

It may be noted that only one of these experiments can be entirely relied upon, and that in this case, after 4 days' exposure to air and $12\frac{1}{4}$ hours of sunshine, there was no result from the inoculation.

These observations, though not in any way conclusive, are in accord with those of Professor Koch,* and they encouraged us to believe that even short exposures of the tubercle-bacillus, even in sputum, to air and light, might render it innocuous.

In the next series of observations, it was determined to allow

* Koch, 'Verhandlungen des Internationalen Medicinischen Congresses' (Berlin, 4th to 9th August, 1890), vol. 1, p. 35. Koch says that for some years it has become known that light could kill bacteria. He alludes, no doubt, to the experiments of Downes and Blunt, Arloing, Roux, and others. Marshall Ward's experiments with the *Bacillus Anthracis* are still more recent. Koch had been able to confirm this with regard to the tubercle-bacillus, of which cultivations exposed to sunlight might be killed in a space of time varying from a few minutes to some hours. When exposed to diffuse daylight in a room they were killed in from five to seven days.

Table II.

Date.	July 20	21	22	23	24	25	26	27	28	29	30	31	Aug. 1	2
Temperature { Maximum.....	96	103	95	95	96	72	70	100	98	93	93	110	105	107
Minimum.....	50	50	50	50	60	53	52	48	56	54	52	50	55	55
Hours of sunshine	3	2	7 $\frac{1}{4}$	1 hour weak shine	2 $\frac{1}{4}$	3 $\frac{1}{2}$	3 $\frac{1}{4}$	1	1 $\frac{1}{2}$	1 $\frac{1}{4}$	4 $\frac{1}{2}$	2 $\frac{1}{2}$	1 $\frac{3}{4}$	1 $\frac{3}{4}$
Total sunshine	12 $\frac{1}{4}$					5 $\frac{3}{4}$								
Results	Exp. 85.—4 days ex- posed. No tuberculosis in 43 days.					Exp. 98.—2 days exposure. Local swelling disappeared in 10 days. No result in 78 days.								
Exp. 97.—Exposed 6 days. No result in 150 days.														

tuberculous sputum to dry (*a*) in air and light, (*b*) in air and darkness, (*c*) in a close cupboard.

Fresh sputum, rich in bacilli, was obtained and exposed in watch glasses. Specimen (*a*) was dry in four days; specimen (*b*) in eight days; and specimen (*c*) in 19 days.

Specimens (*a*) and (*b*) were closed up as soon as they were dry and kept until specimen (*c*) was ready, and then portions of the sputum were scraped off the glasses and inoculated into guinea-pigs directly after scraping. Table III gives the results.

Table III.

No.	
117.	1. } Sputum (<i>a</i>) inoculated subcutaneously into two guinea-pigs,
118.	2. } killed 64 days afterwards, gave well-marked tuberculosis.
119.	3. } Sputum (<i>b</i>), similarly used, showed no results
120.	4. } 53 days after.
126.	5. Sputum (<i>c</i>) gave well-marked tuberculosis 50 days afterwards.

The results of these experiments are somewhat anomalous. The sputum was in rather thick masses and thus dried slowly, and would with difficulty be affected by the natural agents to which they were exposed. This fact would probably account for the continued virulence of sputa of 1 and 2, but the immunity from sputa 3 and 4, after eight days exposure to a current of air in darkness, is hardly likely to be due to this exposure; we can, therefore, draw no decided conclusion from this series of experiments.

In the fourth series of observations, the sputum was spread upon paper, and was thus more rapidly dried at the ordinary temperatures, about 24 hours sufficing. It was then in most cases scraped and thus partly converted into "tuberculous dust" before being exposed to the same conditions as before. In this way it might be expected to be more readily affected by the elements.

An attempt was made to measure the amount of air as well as light, an anemometer and a sunshine-recorder being placed near the sputum exposed at the open window.

Only a rough guess could thus be made as to the quantity of air passing over the sputum, however, for the papers had to be loosely covered with thin gauze to prevent the "dust" from being carried away by the wind, and the anemometer recorded currents in both directions.

Three sets of experiments were made.

1. Papers were placed, to be used for control experiments, in the dark, close cupboard.

2. Papers were placed in the air-shaft of a draught-closet in dim light, pure air only passing through it.

3. Papers were exposed to air and light for three days, February 20, 21, and 22. The rate of air current was about 1,000 ft. per hour, and the sunshine recorded was one hour. Others were exposed for a longer period.

The amount of tuberculous dust was so small that portions of the paper were inserted together with it, under the skin.

Table IV.

First Set of Experiments.

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| No. | |
| 151. | 1. Sputum kept only one day in a closed, dark cupboard, after drying on paper, produced well-marked tuberculosis in 31 days. |
| 191. | 2. Sputum kept under the same conditions, but exposed to a little air for 35 days, produced distinct local tuberculosis in 23 days. |
| 192. | 3. <i>Idem.</i> |

Second Set of Experiments.

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| 160. | 1. Sputum kept in the draught closet for three days in a current of air (about 1,000 cubic feet per hour) in darkness, at the ordinary temperature, gave well-marked tuberculosis in 32 days. |
| 170. | 2. Sputum under exactly the same conditions gave well-marked tuberculosis in 24 days. |

Third Set of Experiments.

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| 156. | 1. Sputum exposed to light for three days, during which there was one hour of sunshine. Ventilation good. Temperature, maximum 50° and minimum 38° F. No tuberculosis after 46 days. |
| 157. | 2. Sputum under the same conditions as the last, except that it had not been reduced to dust, gave the same negative results after 50 days. |
| 189. | 3. Sputum exposed to light for seven days; 15 hours of sunshine; brisk ventilation. Temperature, maximum 88°, minimum 29° F. No tuberculosis after 22 days. |
| 190. | 4. Sputum exposed to light for two days (after being kept dry for four weeks); short exposure to sunshine (not many hours); ventilation slight. Temperature, maximum 60°, minimum 22°. No tuberculosis after 22 days. |

It will be noted that in all the specimens exposed in the dark, tuberculosis was the result, but it must be observed, that in the case of those exposed in the draught-closet, only three days were allowed to pass before they were removed from the influence of the air-current. On the other hand, all the specimens exposed to both air and light, whether for two, three, or seven days, were found to have entirely lost their power for evil.

The specimen exposed for two days only, had, however, been kept for four weeks before being exposed to these influences, and it had thus lost a portion of its virulence.

These researches have an important bearing upon the question of the limits of the infectiveness of tubercle.

It has long been known that the disease is most common in the dirty, ill-drained, ill-ventilated dwellings of the poor, and, even in records intended to prove the contagiousness of phthisis, there are few, if any, of transmission of the disease in clean, well-lighted, well-ventilated houses or hospitals, even those for consumption. Long before Koch's discoveries, and before the disinfection of sputum was practised as it is now, the conveyance of the disease, under these conditions, was recognised by many to be one of the rarest events.

If the results that we have obtained with sputum are confirmed by others, as we trust they will be, they will afford some explanation of these facts.

So far as they extend at present, they show (1) that finely divided tuberculous matter, such as pure cultures of the bacillus, or "tuberculous dust," in daylight, and in free currents of air, is rapidly deprived of virulence, (2) that even in the dark, although the action is retarded, fresh air has still some disinfecting influence, and (3) that in the absence of air, or in confined air, the bacillus retains its power for long periods of time.*

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